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GAS CHROMATOGRAPHIC ANALYSIS OF
HERBICIDES I. RESIDUE ANALYSIS FOR
2,4-D IN SOIL, WATER, AND SEDIMENT

By Donald W. Woodham, G. F. Gardner, and W. F. Barthel
Plant Pest Control Division
Gulfport, Miss.

A method of analysis was needed that would permit residue determination in soil, water, and sediment, of 2,4-D following multiple applications of this herbicide to corn for control of the parasitic chlorophyll bearing plant, witchweed (Striga lutea Lour.).

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Colorimetric methods of analyses for 2,4-D in use until recently (11)¹ lack sensitivity and are cumbersome. Electron capture gas chromatography provides a method with the required sensitivity and is simple and rapid if the residue can be extracted from the substrate and then esterified so that it will pass through the gas chromatograph column. Yip² prepared

¹ Underscored figures in parentheses refer to Selected References at end of report.

² Yip, G. Personal communication on 2,4-D analysis.

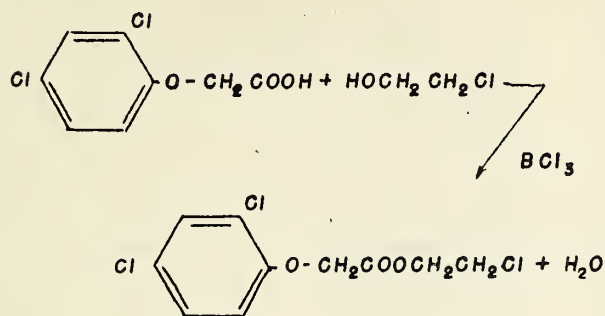
the methyl esters of 2,4-D using diazomethane, in the determination of residues in alfalfa. In our present study, we wished to avoid this reagent because of the hazards associated with its use. Other authors (1, 5, 8, 9) have described methods for preparing methyl esters of fatty acids prior to gas-liquid chromatography (GLC) analysis. These methods usually involve the use of anhydrous methanol containing an acidic or basic catalyst and include a reflux period of 30 minutes to 2 hours.

Mitchell (8, 9) describes the use of boron trifluoride-methanol reagent for the esterification of carboxylic acids. Metcalfe (7) employed this method for the esterification of fatty acids.

Because of the relatively short retention time of the methyl ester (0.65, relative to aldrin), on a 3 percent DC-200 on Gas Chrom-Q, this ester is subject to interference from many extraneous materials (sulfur, for example).

In experimental work with other esterification reagents, the authors found that higher molecular weight reagents formed esters with longer retention times but retained the sensitivity desired in the analysis. One of these esters was the 2-chloroethanol ester of 2,4-D. If 5 ml. of a 10 percent BCl₃ in 2-Chloroethanol

is employed, the esterification proceeds smoothly. The reaction is as follows:



PROCEDURE

Preparation of BCl_3 --2-Chloroethanol reagent, 10 percent BCl_3 by weight

Nine hundred grams of redistilled reagent grade 2-chloroethanol, was weighed into a 2-liter flask and cooled in an ice bath. With the flask still in the bath, BCl_3 was bubbled through a glass tube into the 2-Chloroethanol until 100 grams was dissolved. The solution was agitated with a magnetic stirrer during the entire procedure. A trap was placed in the gas line to prevent liquid from being drawn back into the gas cylinder valve. Preparation of reagent should be carried out in a good fume hood, and the gas should not flow so fast that white fumes emerge from the flask.

Extraction

Soil and Sediment

One hundred fifty grams of soil or sediment was weighed into half-gallon Mason jars; then 300 ml. of redistilled ethyl ether and 5 ml. of a 1:1 solution of H_2SO_4 were added. The pH of the mixture was then tested with pH paper. If the pH was above 3, more sulfuric acid solution was added because the herbicide would not be liberated from the soil, except in the free acid state. The mixture was then rotated on a concentric rotator for 4 hours. The liquid was then decanted into a 500-ml. Erlenmeyer flask and 5 grams of Celite added; the flask was stoppered, shaken well, and the contents filtered through glass wool into a sample bottle.

Water

One to four gallons of water were weighed into a 5-gallon, small-necked bottle. One liter of redistilled ethyl ether and 5 ml. of 1:1 solution of H_2SO_4 were added, the contents swirled to mix the acid, and tested with pH paper. If the pH was above 3, more sulfuric acid was added because the herbicide would not be extracted otherwise. The bottle was closed with a tight fitting cork, covered carefully with aluminum foil, and agitated on a ball mill rotator for 20 minutes. With the bottle upright, the ether was then removed by slowly running tap water into the bottle through one delivery tube in a two-hole stopper. This delivery tube extended below the water level in the bottle. The other delivery tube in the two-hole stopper extended just below the stopper and lead to a liter-graduated cylinder. Both delivery tubes were made of stainless steel to prevent breakage. The water slowly running into the bottle floated the solvent out of the bottle for collection in the graduate. When the first drops of water appeared, the graduate was removed, and the contents was transferred to an Erlenmeyer flask after recording the volume. A small quantity (about 5 grams) of anhydrous sodium sulfate was added to take up any water, and the contents of the flask concentrated to somewhat less than 50 ml. through a three-bulb Snyder column. The liquid was then transferred to a 50-ml. graduate; made to 50 ml. volume, and stored in a glass can sample bottle after stoppering with a crown seal covered with aluminum foil. From this point on, the analysis was the same as for soil or sediment.

Esterification Procedure

Fifty milliliters of the ether extract representing 25 grams of soil or sediment or 3.76 kg. water was aliquoted into 250-ml. Erlenmeyer flasks and concentrated by evaporating the solvent on a water bath through three-bulb Snyder columns to about 5 ml. Fifty milliliters of redistilled hexane was added, and the solution again concentrated to about 5 ml. Five milliliters of the boron trichloride reagent was added, mixed, and heated on a hot water bath for 10 minutes swirling occasionally at a temperature of about 70°C . Fifty milliliters of distilled water and 50 ml. of redistilled pentane

were added to the Erlenmeyer flask; the mixture was then transferred to a 250-ml. separatory funnel, shaken, allowed to equilibrate, and the water layer drawn off into a second 250-ml. separatory funnel. The water layer was washed two more times with redistilled pentane, using 30 ml. of pentane for each wash, and was then discarded. The three pentane fractions (ca. 110 ml.) were combined in 250-ml. Erlenmeyer flasks, and a small amount of anhydrous Na_2SO_4 was added and concentrated to approximately 10 ml. through three-bulb Snyder columns on a warm water bath. The pentane extracts were then transferred to 15-ml. centrifuge tubes; the volume was adjusted to exactly 10 ml., and a small amount of anhydrous Na_2SO_4 added for drying.

Analysis

Five microliters of the pentane solution were injected into the gas chromatograph with a 3 percent DC-200 on 100-120 mesh gaschrom-Q in a glass column. Residues were calculated by comparison with 2,4-D standards which were esterified and injected as described. Instrumental operating conditions follow.

Instrument: F & M Model 810 Research Gas Chromatograph, dual column with dual electron capture detectors (tritium source).

Recorder: Honeywell "Electronic 16," 11-inch strip chart recorder.

Column: 6-foot all glass columns, 4.0 mm. i.d.

Carrier and purge gas: 5 percent Methane, 95 percent Argon.

Flow rates:

Carrier gas - 90 ml./min.

Tank pressure - 60 p.s.i.g.

Purge - 40 ml./min.

Temperatures:

Column - 170°C.

Detector - 200°C.

Injection port - 240°C.

Chart speed: 15 inches/hour.

Range 10: Attenuation 64 (2.5×10^{-9} a.)

RESULTS AND DISCUSSION

Soil, water, and sediment samples were analyzed by the extraction and esterification procedure described in this report. These re-

sults were compared to those obtained by esterifying aliquots of the same samples to the methyl ester. The 2-chloroethanol ester of 2,4-D increased in retention time (2.18, relative to aldrin on the QF-1 column), so as to move away from extraneous peaks appearing at the beginning of the chromatogram (figs. 1 and 2). The methyl ester of 2,4-D is included for comparison.

Soil, water, and sediment samples were fortified with known amounts of 2,4-D acid, extracted and esterified to 2-chloroethanol ester, then analyzed by GLC. Recovery values were calculated by comparing peak heights from the 2-chloroethanol ester of 2,4-D in the samples with a standard of 2,4-D esterified in the same manner (tables 1, 2, and 3). Duplicate aliquots were esterified to the methyl ester and analyzed

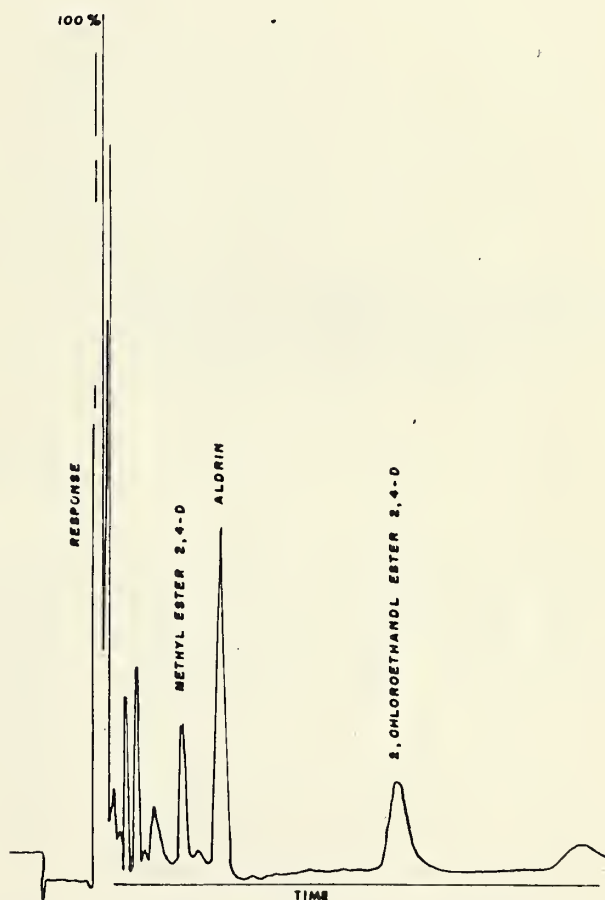


Figure 1.--Chromatographic trace of sediment sample fortified with aldrin, 0.43 nanogram (ng.); methyl ester 2,4-D, 1.29 ng.; and 2-chloroethanol ester 2,4-D, 2.14 ng. on 5 percent QF-1 column.

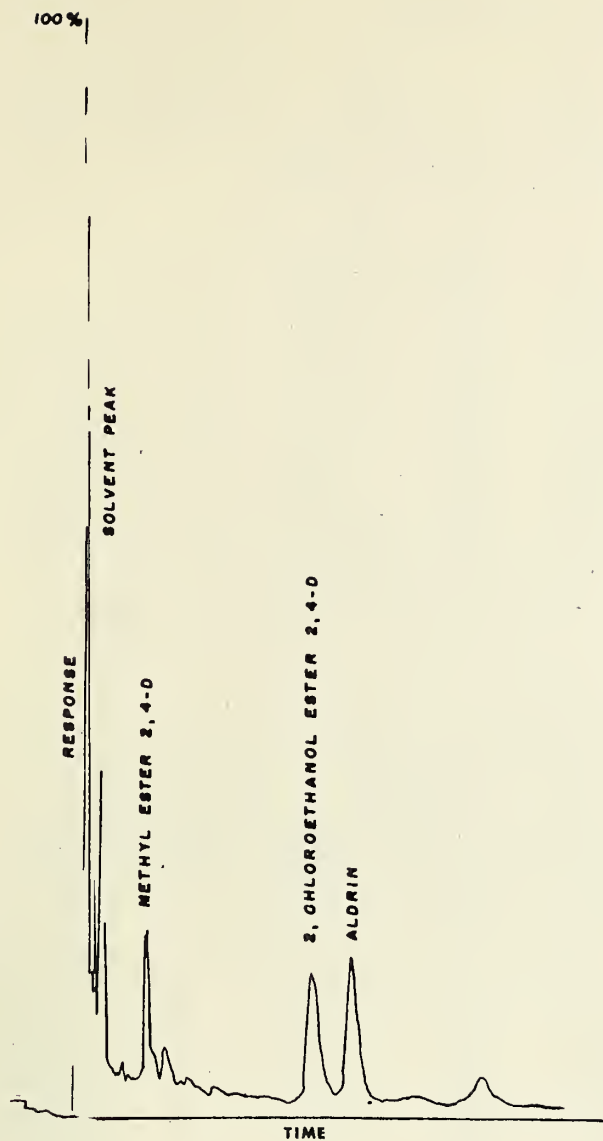


Figure 2.--Chromatographic trace of sediment sample fortified with aldrin, 0.43 nanogram (ng.); methyl ester 2,4-D, 1.29 ng.; and 2-chloroethanol ester 2,4-D, 2.14 ng. on DC-200 column.

by GLC as for the 2-chloroethanol ester. The higher recovery value obtained from the methyl ester of 2,4-D is due to extraneous material in the sediment sample which had the same retention time as the methyl ester. The value (table 2) has been corrected for this material and the material calculated on the basis of the 2,4-D methyl ester standard. There were no interfering peaks for the 2-chloroethanol ester of 2,4-D (fig. 1) in sediment samples.

We cannot fully explain the low recovery values obtained from the soil samples. Apparently the soil adsorbed more of the 2,4-D

than of other types of substrates. The recovery values for the 2-chloroethanol ester were as high as for the methyl ester on these soil samples and was subject to less interferences from other substances present in the soil extract.

Work is now in progress to evaluate the 2-chloroethanol method for analysis of other herbicides (2, 4, 5-T, MCPA, and similar formulations). Studies have not been completed for this reagent in the analysis of different phenoxyacetic acid herbicides in various substrates such as crops and biological samples.

Table 1.--2,4-D recovery soil ¹

Sample No.	2,4-D acid added	Recovered		Recovered		Average recovery	
		Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester
	<u>P.p.m.</u>	<u>P.p.m.</u>	<u>P.p.m.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>
1.....	0.33	0.22	0.23	66.7	69.7	--	--
2.....	.33	.19	.19	57.6	57.6	--	--
3.....	.33	.23	.21	69.7	63.6	64.7	63.6
4.....	.67	.39	.37	58.2	55.2	--	--
5.....	.67	.37	.38	55.2	56.7	--	--
6.....	.67	.37	.33	55.2	49.3	56.2	53.7
7.....	1.00	.54	.48	54.0	48.0	--	--
8.....	1.00	.54	.44	54.0	44.0	--	--
9.....	1.00	.53	.41	53.0	41.0	53.7	44.3

¹ Determinations made by electron capture with 3 percent DC-200 column.

Table 2.--2,4-D recovery, sediment ¹

Sample No.	2,4-D acid added	Recovered		Recovered		Average recovery	
		Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester
	<u>P.p.m.</u>	<u>P.p.m.</u>	<u>P.p.m.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>
1.....	0.33	0.35	0.29	106.1	87.8	--	--
2.....	.33	.39	.40	121.3	121.3	--	--
3.....	.33	.34	.32	97.0	97.0	111.3	102.0
4.....	.67	.91	.63	135.8	94.5	--	--
5.....	.67	.75	.57	111.9	85.5	--	--
6.....	.67	.85	.60	126.8	89.5	127.8	89.8
7.....	1.00	1.00	.78	100.0	78.0	--	--
8.....	1.00	1.07	.78	107.0	78.0	105.5	78.0

¹ Determinations made by electron capture with 3 percent DC-200 column.

Table 3.--2,4-D recovery, water ¹

Sample No.	2,4-D acid added	Recovered		Recovered		Average Recovery	
		Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester	Methyl ester	2-Chloroethanol ester
	<u>P.p.b.</u>	<u>P.p.b.</u>	<u>P.p.b.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>	<u>Pct.</u>
1.....	5,32	4,81	4,74	90,4	89,1	--	--
2.....	5,32	3,81	3,66	71,6	68,8	--	--
3.....	5,32	4,07	4,44	76,5	83,5	79,5	80,5
4.....	10,64	8,69	8,57	81,7	80,5	--	--
5.....	10,64	8,12	10,26	76,3	96,4	--	--
6.....	10,64	10,52	10,26	98,9	96,4	85,6	91,1
7.....	15,96	10,03	14,39	62,8	90,2	--	--
8.....	15,96	13,96	13,22	87,5	82,8	--	--
9.....	15,96	13,68	11,82	85,7	74,1	78,7	82,4

¹ Determinations made by electron capture with 5 percent QF-1 column.

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